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Effects of nitrogen form on growth, CO_2 assimilation, chlorophyll fluorescence, and photosynthetic electron allocation in cucumber and rice plants*

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Abstract: Cucumber and rice plants with varying ammonium (NH_4^+) sensitivities were used to examine the effects of different nitrogen (N) sources on gas exchange, chlorophyll (Chl) fluorescence quenching, and photosynthetic electron allocation. Compared to nitrate (NO_3^-)-grown plants, cucumber plants grown under NH_4^+-nutrition showed decreased plant growth, net photosynthetic rate, stomatal conductance, intercellular carbon dioxide (CO_2) level, transpiration rate, maximum photochemical efficiency of photosystem II, and O_2-independent alternative electron flux, and increased O_2-dependent alternative electron flux. However, the N source had little effect on gas exchange, Chl *a* fluorescence parameters, and photosynthetic electron allocation in rice plants, except that NH_4^+-grown plants had a higher O_2-independent alternative electron flux than NO_3^--grown plants. NO_3^- reduction activity was rarely detected in leaves of NH_4^+-grown cucumber plants, but was high in NH_4^+-grown rice plants. These results demonstrate that significant amounts of photosynthetic electron transport were coupled to NO_3^- assimilation, an effect more significant in NO_3^--grown plants than in NH_4^+-grown plants. Meanwhile, NH_4^+-tolerant plants exhibited a higher demand for the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) for NO_3^- reduction, regardless of the N form supplied, while NH_4^+-sensitive plants had a high water-water cycle activity when NH_4^+ was supplied as the sole N source.

Key words: Nitrogen form, Photosynthetic electron allocation, Alternative electron flux, Nitrate reductase
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1 Introduction

Nitrogen (N) is one of the most important nutrients affecting the growth, development, yield, and fruit quality of plants (Fernandes and Rossiello, 1995; Gerendás *et al.*, 1997). It is required in the greatest quantities at each stage of plant growth during which N level markedly affects the amount of Rubisco content, and therefore photosynthesis (Evans and Terashima, 1988; Evans, 1989). In addition to the amount supplied, the form of N available has significant effects on the growth and photosynthesis of plants (Cramer and Lewis, 1993), and preference for the N sources nitrate (NO_3^-) and ammonium (NH_4^+) varies between plant species (Baker and Mills, 1980). For example, NH_4^+ was reported to be superior to NO_3^- for the growth of rice and conifer (Gerendás *et al.*, 1997; Britto and Kronzucker, 2002; 2004), whereas some plant species such as cucumber, tomato, eggplant, maize, barley, wheat, and bean prefer NO_3^-

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to NH_4^+ nutrition (Bloom *et al.*, 1989; Kafkafi, 1990; Cramer and Lewis, 1993; Claussen and Lenz, 1995; Roosta and Schjoerring, 2007). Meanwhile, some plant species show enhanced growth when cultivated in both NO_3^- and NH_4^+ conditions, compared with growth under sole NO_3^- and NH_4^+ conditions (Errebhi and Wilcox, 1990). Furthermore, environmental conditions, such as temperature, light intensity, soil water content, pH, and ions in the growth medium, also affect plant sensitivity to NO_3^- and NH_4^+ nutritions (Kotsiras *et al.*, 2005). For example, the presence of potassium (K^+) may alleviate NH_4^+ toxicity (Szczerba *et al.*, 2006). The management of the amount and form of N in the nutrient solutions of crops is a complex and important part of successful crop production system.

In the last two decades, a large number of studies have focused on the effects of NO_3^- and NH_4^+ nutritions on plant photosynthetic physiology, which is one of the most important metabolic processes affected by N form (Geiger *et al.*, 1999; Guo *et al.*, 2002; Lopes *et al.*, 2004; Lopes and Araus, 2006). The effect of N form on photosynthesis is manifested in differences in the stomatal conductance, and the intercellular carbon dioxide (CO_2) partial pressures of NO_3^-- and NH_4^+-supplied plants, but the results are not consistent between different plant species. For example, decreases in CO_2 assimilation due to stomatal closure were observed in wheat and barley when NH_4^+ was present (Lopes *et al.*, 2004; Lopes and Araus, 2006), but plants such as French bean and tobacco supplied with NH_4^+ had a higher CO_2 assimilation rate and stomatal conductance than those supplied with NO_3^- (Geiger *et al.*, 1999; Guo *et al.*, 2002). N form also has different effects on the Calvin cycle activity, indicated by the Rubisco content or capacities of Rubisco carboxylation and ribulose-1,5-bisphosphate regeneration. Raab and Terry (1994; 1995) showed that the leaf Rubisco content in NH_4^+-grown sugar beet plants was significantly higher than that in NO_3^--grown plants, which was similar to those found in tobacco plants by Terce-Laforgue *et al.* (2004).

Although the effects of N form on stomatal factors and the Calvin cycle in the photosynthesis process have been extensively investigated, relatively few studies have focused on the relationship between photosynthetic electron transport and electron utilization in photosystem II (PSII) in plants with different sensitivities to NO_3^- and NH_4^+ nutritions. It is well known that light energy is mainly used by the photosynthetic linear electron transport to produce assimilatory powers [adenosine triphosphate (ATP) and reduced form of nicotinamide adenine dinucleotide phosphate (NADPH)] for CO_2 assimilation. When plants absorb energy that exceeds their photosynthetic capacity, they activate some physiologic processes to protect the chloroplast from photodamage, such as xanthophyll cycle-dependent heat dissipation from antennae in PSII, the D1 repair cycle, photorespiration, N assimilation, and the operation of the water-water cycle (Osmond and Grace, 1995; Demmig Adams and Adams, 1996; Zhou *et al.*, 2004; Kanervo *et al.*, 2005; Brück and Guo, 2006; Wang *et al.*, 2008). There are marked differences in the photo-energy consumption and the supply of reducing equivalents between NO_3^- and NH_4^+ nutritions. In the processes of NO_3^- uptake, transport, reduction, and assimilation, almost 32 mol photons per mol N were consumed, while the energy cost for NH_4^+ is only 9.45 mol photons per mol N (Raven, 1985). Accordingly, NO_3^- assimilation may represent a considerable additional reductant sink increasing NADPH consumption (Guo *et al.*, 2007).

Substantial differences in electron utilization have been found under sole NO_3^- and NH_4^+ conditions. However, allocation of photosynthetic electrons to CO_2, O_2, and NO_3^- reduction and cyclic electron flow, as influenced by N form, remain poorly understood. In this work, we studied the relationship between the characteristics of NO_3^-/NH_4^+-sensitivity and photosynthetic electron utilization by calculating the electron flux in PSII.

2 Materials and methods

2.1 Plant materials and treatments

Cucumber (*Cucumis sativus* L. cv. Jinyou No. 30) seeds were first sown in a substrate containing a mixture of soil and perlite (1:1, v:v) in 72-cell plastic trays. Groups of six seedlings at the two-leaf stage were transplanted into 15-L plastic containers filled with a nutrient solution comprising the following nutrients (in units of mmol/L): 2.0 $Ca(NO_3)_2$, 2.0 KNO_3, 2.5 KH_2PO_4, 2.0 $MgSO_4$, 29.6×10^{-3} H_3BO_3, 10×10^{-3} $MnSO_4$, 50×10^{-3} Fe-Na_2EDTA, 1.0×10^{-3} $ZnSO_4$, 0.05×10^{-3} H_2MoO_4, and 0.95×10^{-3} $CuSO_4$. At the same time, rice (*Oryza sativa* L. cv. Taiyou No. 1) seeds were germinated in Petri dishes moistened with distill water. Five to seven days later, six seedlings were transplanted into the same containers (15 L) containing a nutrient solution comprising the following nutrients (in units of mmol/L): 3.0 NH_4NO_3, 0.6 Na_2HPO_4, 0.3 K_2SO_4, 0.4 $MgCl_2$, 0.2 $CaCl_2$, 45×10^{-3} Fe-EDTA, 50×10^{-3} H_3BO_3, 9×10^{-3} $MnSO_4$, 0.7×10^{-3} $ZnSO_4$, 0.3×10^{-3} $CuSO_4$, and 0.1×10^{-3} H_2MoO_4. The nutrient solution was changed once a week, and its pH was adjusted with 1 mol/L HCl to 6.5 for cucumber plants and 5.5 for rice plants. The environmental conditions in the greenhouse were as follows: a 12-h photoperiod, temperatures of 28/22 °C (day/night), and an average photosynthetic photon flux density (PPFD) of 500 μmol/(m^2·s).

The treatments, which differed only in the source of N, were imposed at the three- to four-leaf stages for cucumber and at the four-leaf stage for rice. Two N treatments at constant N concentrations were administered: 6.0 mmol/L N as NO_3^- only or as NH_4^+ only for cucumber and rice plants, respectively. The solutions were renewed every two days to minimize variations in pH and nutrient concentrations. Seven days after the treatments were started, gas exchange and chlorophyll (Chl) *a* fluorescence analyses were carried out. The roots and shoots of cucumber and rice plants were then harvested and oven-dried at 80 °C for three days for the determination of the dry weight of plants. Meanwhile, the leaves were also sampled, snap frozen in liquid nitrogen, and stored at −86 °C until their use for the analysis of NO_3^- reductase activity. Each treatment had 18 plants with four replicates.

2.2 Gas exchange and Chl *a* fluorescence measurements

Gas exchange and Chl *a* fluorescence were determined simultaneously on the 2nd leaf with an LI-6400 portable open gas exchange system equipped with an LI-6400 leaf chamber Chl fluorometer attachment (Li-Cor, Lincoln, NE, USA). Leaf net photosynthesis rate (P_n), stomatal conductance (g_s), intercellular CO_2 concentration (c_i), and transpiration rate (T_r) were measured at 25 °C with PPFD of 500 μmol/(m^2·s) and CO_2 concentration of 360 μmol/mol. Meanwhile, steady state fluorescence yield (F_s) and light-adapted maximum fluorescence (F'_m) were recorded for the calculations of the parameters including the quantum efficiency of PSII (Φ_{PSII}), photochemical quenching coefficient (q_P), and the efficiency of excitation capture by open PSII centers (F'_v/F'_m) (Zhou *et al.*, 2004). The plants were then placed in a dark room for 20 min to determine the maximum photochemical efficiency of PSII (F_v/F_m) (Zhou *et al.*, 2004).

2.3 Electron transport analyses

The total electron flux in PSII (J_{PSII}) was measured according to the methods of Miyake and Yokota (2000), and can be calculated as: $J_{PSII}=\Phi_{PSII}\times\alpha\times$PPFD, where α is the product of the absorbance coefficient and ratio of allocation of excitation energy to PSII. As Miyake and Yokota (2000) suggest, J_{PSII} can be divided into four components, which can be determined by measuring P_n and chlorophyll parameters under both 21% and 2% O_2 conditions. The four components are electron flux for photosynthetic carbon reduction (J_c), electron flux for photo-respiratory carbon oxidation (J_o), O_2-dependent alternative electron flux [J_a (O_2-dependent)], and O_2-independent alternative electron flux [J_a (O_2-independent)].

2.4 Nitrate reductase (NR) activity estimation

The activity of NR in the leaves was measured according to Li *et al.* (2006) and was expressed as nanomoles of NO_2^- per gram fresh weight (FW) per minute.

2.5 Statistical analysis

All the measurements were repeated at least four times from different individual plants. Data were statistically analyzed using analysis of variance (ANOVA), and tested for significant treatment differences using the Duncan's multiple range test at $P<0.05$ with Origin 7.5 Software (OriginLab, Northampton, MA, USA).

3 Results

3.1 Effect of N form on plant biomass

Cucumber plants supplied with NH_4^+-N showed slower growth compared to those grown with NO_3^--N. By the final harvest, 7 d after onset of treatment, the dry mass of shoot, root, and total plant for cucumber NO_3^--grown plants was 79.4%, 144.8%, and 138.7% higher, respectively, than that of NH_4^+-grown plants (Table 1). In contrast, the N form had little effect on rice plant growth, and there were no significant differences in shoot, root, and total plant biomass accumulations 7 d after the treatment.

Table 1 Effects of different nitrogen forms on dry mass of shoot, root, and total plant

Species	N form	Dry mass (g/plant)		
		Shoot	Root	Total plant
Cucumis sativus	NO_3^-	3.05±0.09a	0.39±0.03a	4.44±0.11a
	NH_4^+	1.70±0.14b	0.16±0.01b	1.86±0.09b
Oryza sativa	NO_3^-	11.94±0.69a	4.83±0.55a	16.77±0.32a
	NH_4^+	14.19±2.46a	4.70±0.79a	18.89±1.77a

Values are expressed as mean±SD (n=4). Significant differences (P<0.05) between treatments within the same species are indicated by different letters

3.2 Effect of N form on gas exchange

Gas exchange analysis showed that the N form had significant effects on gas exchange in cucumber plants, but not in rice plants. As shown in Fig. 1, NH_4^+ nutrition decreased P_n by 38.5%, compared with the NO_3^- nutrition in cucumber plants. At the same time, lower g_s, c_i, and T_r were found in NH_4^+-grown plants, which decreased by 64.3%, 14.1%, and 43.1%, respectively, compared with their NO_3^--grown plants. However, the N form had a minor effect on rice plants, since the changes in these parameters were almost independent of the N form supplied.

3.3 Effect of N form on Chl *a* fluorescence

The changes in F_v/F_m, Φ_{PSII}, q_P, and F_v'/F_m' as influenced by N form in cucumber and rice leaves are shown in Fig. 2. For cucumber leaves, the values of F_v/F_m value declined from 0.82 for the NO_3^--grown plants to 0.75 for the NH_4^+-grown plants, indicating that a slight photo-inhibition occurred in NH_4^+-grown plants. Significant reductions in Φ_{PSII} and q_P were also found in NH_4^+-grown cucumber leaves, which decreased by 22.2% and 20.3%, respectively, compared with their NO_3^--treated leaves. However, similar values of F_v'/F_m' were observed in cucumber plants between NO_3^- and NH_4^+ treatments, which

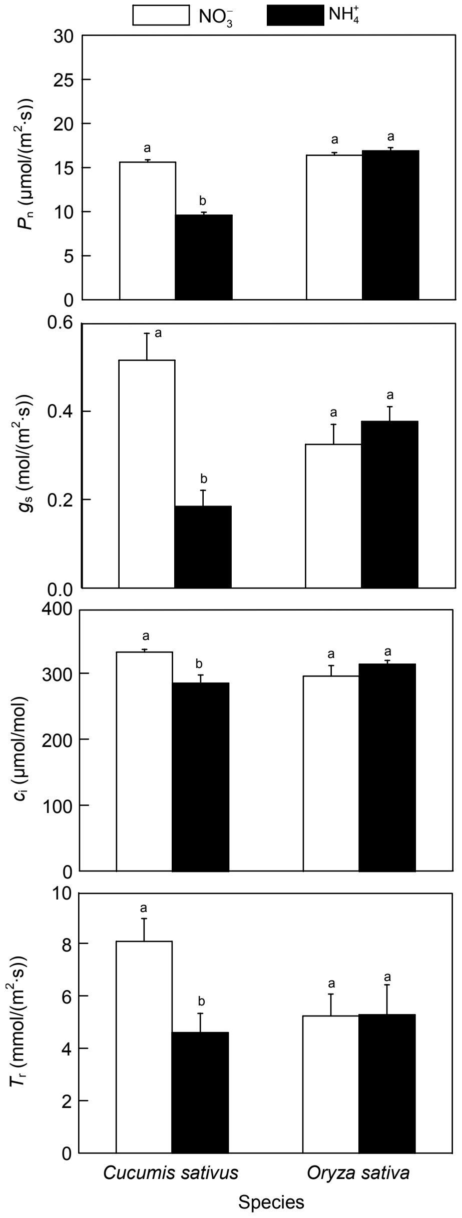

Fig. 1 Effects of different nitrogen forms on CO_2 assimilation rate (P_n), stomatal conductance (g_s), intercellular CO_2 (c_i), and transpiration rate (T_r)

Values are expressed as mean±SD (n=4). Significant differences (P<0.05) between treatments within the same species are indicated by different letters suggested that the decline of $\Phi_{\rm PSII}$ in cucumber plants was due to declines of $q_{\rm P}$ alone, with no contribution from $F'_{\rm v}/F'_{\rm m}$. In sharp contrast, there was no significant effect of the N form on the Chl *a* fluorescence parameters $\Phi_{\rm PSII}$, $q_{\rm P}$, and $F'_{\rm v}/F'_{\rm m}$ in rice.

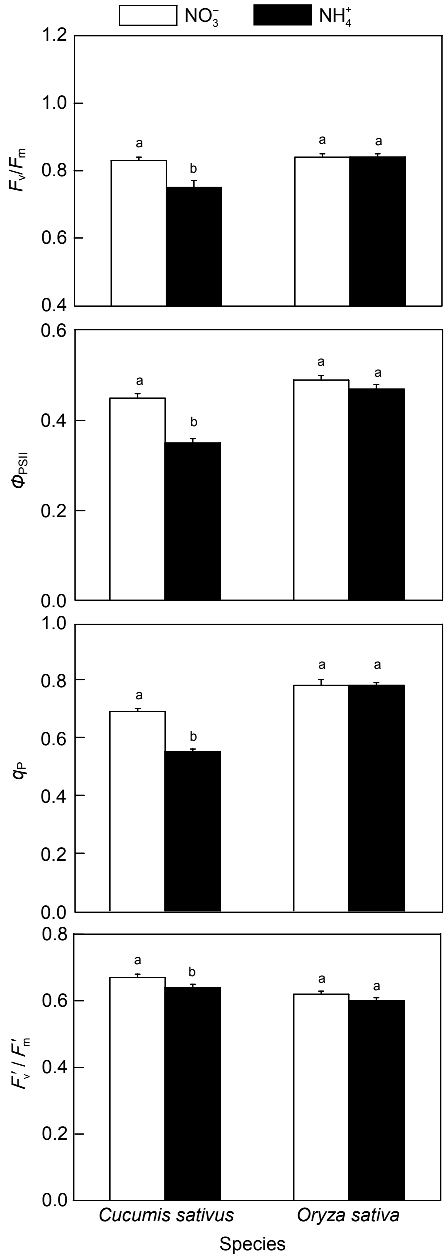

Fig. 2 Effects of different nitrogen forms on the maximum photochemical efficiency of PSII ($F_{\rm v}/F_{\rm m}$), the quantum efficiency of PSII ($\Phi_{\rm PSII}$), photochemical quenching coefficient ($q_{\rm P}$), and the efficiency of excitation capture by open PSII centers ($F'_{\rm v}/F'_{\rm m}$)

Values are expressed as mean±SD (n=4). Significant differences ($P<0.05$) between treatments within the same species are indicated by different letters

3.4 Effect of N form on allocation of electron flux in PSII

For NO_3^--grown cucumber plants, $J_{\rm c}$, $J_{\rm o}$, $J_{\rm a}$ (O_2-dependent), and $J_{\rm a}$ (O_2-independent) accounted for 73.0%, 13.4%, 6.1%, and 7.5%, respectively, of the total electron flux in PSII ($J_{\rm PSII}$) (Table 2). Compared to NO_3^--grown cucumber plants, $J_{\rm PSII}$, $J_{\rm c}$, $J_{\rm o}$, and $J_{\rm a}$ (O_2-independent) decreased by 19.8%, 22.8%, 23.5%, and 45.9%, respectively, while $J_{\rm a}$ (O_2-dependent) increased by 56.1% in NH_4^+-grown cucumber plants. $J_{\rm a}$ (O_2-independent) accounted for 54.9% of the total $J_{\rm a}$ for NO_3^--grown cucumber plants compared to 29.6% for the NH_4^+-grown cucumber plants. Interestingly, the N form had negligible effects on the allocation of electron flux in PSII except that $J_{\rm a}$ (O_2-independent) for NO_3^--grown rice plants was 92.8% higher than that for NH_4^+-grown rice plants. At last, $J_{\rm a}$ (O_2-independent) accounted for 78.7% of the total $J_{\rm a}$ for NO_3^--grown rice plants as compared to 53.3% for the NH_4^+-grown rice plants.

3.5 Effect of N form on NR activity

Although cucumber and rice plants received the same N level, the leaves of rice plants exhibited higher NR activity when grown with either N form. Meanwhile, NR activity was consistently higher in leaves of the NO_3^--grown plants compared to those of NH_4^+-grown plants (Fig. 3). Interestingly, NR activity was near zero in leaves of NH_4^+-grown cucumber

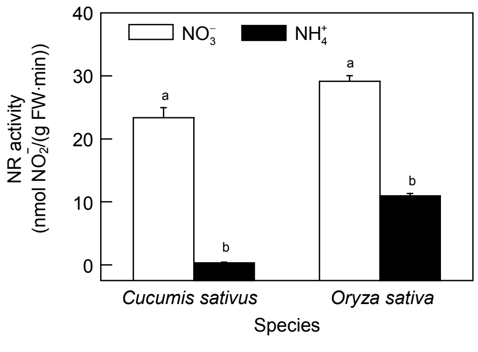

Fig. 3 Effects of different nitrogen forms on nitrate reductase (NR) activity in leaves

Values are expressed as mean±SD (n=4). Significant differences ($P<0.05$) between treatments within the same species are indicated by different letters

Table 2 Effects of different nitrogen forms on the total electron flux in PSII (J_{PSII}), electron flux for photosynthetic carbon reduction (J_c), electron flux for photo-respiratory carbon oxidation (J_o), O_2-dependent alternative electron flux [J_a (O_2-dependent)], and O_2-independent alternative electron flux [J_a (O_2-independent)] in cucumber and rice leaves

Species	N form	J_{PSII} (μmol e/(m^2·s))	J_c (μmol e/(m^2·s))	J_o (μmol e/(m^2·s))	J_a (O_2-dependent) (μmol e/(m^2·s))	J_a (O_2-independent) (μmol e/(m^2·s))
Cucumis sativus	NO_3^-	106.1±5.8a	77.4±5.0a	14.2±0.3a	6.5±1.0b	7.9±0.3a
	NH_4^+	85.1±2.9b	59.8±2.6b	10.9±0.9b	10.2±0.9a	4.3±0.7b
Oryza sativa	NO_3^-	111.9±2.9a	80.1±2.1a	18.9±1.3a	2.8±0.6a	10.2±1.3a
	NH_4^+	107.4±2.4a	80.0±1.9a	17.5±0.5a	4.6±0.8a	5.3±0.8b

Values are expressed as mean±SD (n=4). Significant differences (P<0.05) between treatments within the same species are indicated by different letters

plants, but high NR activity was observed in NH_4^+-grown rice plants. Finally, NR activity in the NO_3^--grown leaves was 72.6-fold and 1.7-fold higher than that in the NH_4^+-grown leaves for cucumber and rice plants, respectively.

4 Discussion

Our results provide clear evidence of significant species variation in the susceptibility of plant growth to the N form supplied. In this study, a greater dry mass of shoot, root, and total plant was observed in cucumber under growth conditions using NO_3^- as the sole N source, compared to NH_4^+ as the sole N source (Table 1). In contrast, there was no significant difference in biomass accumulation in rice between NO_3^- and NH_4^+ nutritions. Accordingly, rice is an NH_4^+-tolerant plant species, while cucumber is an NH_4^+-sensitive plant species. This is in agreement with earlier studies that cucumber plants are well adapted to NO_3^- as the predominant N form (Roosta and Schjoerring, 2007), and that rice plants appear to be adapted to either NH_4^+ or NO_3^- as the predominant N form (Britto and Kronzucker, 2002). Studying the responses of CO_2 assimilation, Chl *a* fluorescence, and photosynthetic electron allocation to NO_3^- or NH_4^+ nutrient will help in revealing the species differences in NO_3^-/NH_4^+ sensitivity.

Photosynthesis is one of the primary processes responsible for plant growth, and photosynthetic metabolism will be finely regulated to adapt growth under different N source conditions (Claussen and Lenz, 1995; Brück and Guo, 2006). In fact, significant effects of the N form on photosynthesis were found in cucumber plants. As shown in Fig. 1, NH_4^+-grown cucumber plants had a significantly lower P_n compared with NO_3^--grown cucumber plants, accompanied by lower g_s and c_i. This is in agreement with earlier observations that NH_4^+ decreased stomatal conductance and transpiration in French beans (Guo *et al.*, 2002) and tobacco (Lu *et al.*, 2005). However, the N source had no significant effect on the photosynthesis parameters in rice plants in other studies (Guo *et al.*, 2008; Li *et al.*, 2009).

Similar to the changes observed with gas exchange analysis, a higher Φ_{PSII} value was found in cucumber plants grown with the NO_3^--N source, but no significant differences in Φ_{PSII} were observed between NO_3^--N and NH_4^+-N sources in rice. Increases in the rate of photosynthetic linear electron transport were also found in barley, pea, and wheat when grown with NO_3^- rather than NH_4^+ as the sole N source (Bloom *et al.*, 1989; 2002; de la Torre *et al.*, 1991). It is worthwhile to note that cucumber plants experienced photo-inhibition when they were grown with NH_4^+ as the N source, as has been observed in the NH_4^+-grown French bean plants (Zhu *et al.*, 2000). Several studies have found that NH_4^+-sensitive plant species exhibit increased photo-respiration to protect the plants from the effects of photodamage (Zhu *et al.*, 2000). However, our study showed that photo-respiration (i.e., J_o) was independent of the N source, suggesting that other photo-protection mechanisms are involved in NH_4^+-sensitive plants.

It has been well established that excess NADPH in the light reaction, which is not completely used during CO_2 assimilation, is either exported from the chloroplasts or consumed by the reductant sinks (e.g., leaf NO_3^- assimilation or water-water cycle) (Brück and Guo, 2006). Previous studies have indicated that a substantial portion of photosynthesis or respiration electron transport generates reducing equivalents for NO_3^- reduction rather than for carbon fixation (Bloom *et al.*, 1989; Noctor and Foyer, 1998; Lewis *et al.*, 2000), and thus, NO_3^- reduction may represent a considerable reductant sink (Brück and Guo, 2006). However, this sink for NADPH would not be expected when NO_3^- is absent from the leaves, and it would decrease with a decrease in the NR reaction. Although J_a (O_2-independent) driven by NO_3^- reduction has been suggested as an important sink for electrons in chloroplasts (Miyake and Yokota, 2000; Zhou *et al.*, 2004), few studies have attempted to quantitate this contribution.

With the methods developed by Miyake and Yokota (2000), we divided the total J_{PSII} into J_c, J_o, J_a (O_2-dependent), and J_a (O_2-independent). In this model, J_a (O_2-dependent) and J_a (O_2-independent) were most driven by the water-water cycle and NO_3^- reduction, respectively (Miyake and Yokota, 2000). J_a (O_2-independent) accounted 5.0% to 7.5% of the total J_{PSII} for plants fed with NO_3^--N and NH_4^+-N in cucumber, and 4.9% to 9.1% for plants fed with NO_3^--N and NH_4^+-N in rice (Table 2). It is interesting to note that J_a (O_2-independent) was detected in both species plants fed with NH_4^+-N and there is a close relation between NR activity and J_a (O_2-independent) (Table 2; Fig. 3). It is likely that NH_4^+ absorbed was converted to NO_3^- in roots or shoots during growth since this reaction is frequent in plants. It is also possible that other O_2-independent metabolism pathway was also involved in the utilization of NADPH and ATP. Taken together, these results suggest that NR-driven J_a (O_2-independent) works as an important NADPH sink in electron transport during photosynthesis.

When the combined operation of the electron-consuming processes described above fails to dissipate safely, all of the electrons generated by PSII and the water-water cycle activity [J_a (O_2-dependent)] will increase. In this study, a higher J_a (O_2-dependent) for NH_4^+-grown cucumber plants was observed compared to NO_3^--grown plants, which indicates a greater rate of electron transport to O_2. Compared with cucumber plants, rice exhibited a lower proportion of J_a (O_2-dependent), irrespective of the N form, and almost the same values in J_a (O_2-dependent) were found in NO_3^- and NH_4^+ nutrition treatments. Normally, enhancement of reactive oxygen species (ROS) generation was correlated with the increase of J_a (O_2-dependent), as has been found in chilled cucumbers (Zhou *et al.*, 2004). In fact, impaired plant growth together with photoinhibition occurred in cucumber leaves when grown with NH_4^+ as the sole N source. It is likely that NH_4^+-induced ROS generation leads to photo-inhibition and impairs plant growth. Further work on NH_4^+-N source and the associated N metabolism in roots and leaves will help us to elucidate how photo-inhibition and the water-water cycle activity are altered by the N form.

In summary, we compared rice and cucumber grown with NO_3^- and NH_4^+ as the N sources to examine the interaction between N assimilation and photosynthetic electron allocation. Significant amounts of photosynthetic electron transport appeared to be coupled to NO_3^- assimilation, and this was more significant in NO_3^--grown plants than in NH_4^+-grown plants. Meanwhile, NH_4^+-tolerant plants exhibited a higher demand for NADPH for NO_3^- reduction regardless of the N forms supplied, while NH_4^+-sensitive plants had a relatively high water-water cycle activity when NH_4^+ was supplied as the sole N source.

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